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Research Article
Level of Heavy Metals in Two Highly Consumed Fish Species at District Lower Dir, Khyber Pakhtunkhwa, Pakistan

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Abstract
The current study was designed to assess heavy metals' concentration in muscle tissues of two Chinese carps, common carp (*Cyprinus carpio*) and silver carp (*Hypophthalmichthys molitrix*), available to consumers in markets at district Lower Dir, Khyber Pakhtunkhwa, Pakistan. Fish specimens were collected from three main markets in the study area namely; Chakdara, Timergara and Khall. Heavy metals including; manganese (Mn), lead (Pb), iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd) and cobalt (Co) were investigated using atomic absorption spectrophotometer. Cobalt was not detected in any of the fish specimens while the rest of the metals were lying within the permissible limits suggested by FAO/WHO and ITS for food/fish consumption. The results showed a statistically significant ($p<0.05$) difference between both species with respect to the concentration of the accumulated heavy metals. In common carp, the heavy metal accumulation was in order of Fe>Mn>Zn>Pb>Cu>Cd, while in silver carp the order was Fe>Mn>Zn>Pb>Cd>Cu. Higher concentration of Pb, Mn, Zn, Cu and Cd was recorded in muscle of common carp while the concentration of Fe was higher in silver carp, indicating higher potential of accumulation of heavy metals in common carp. Statistically significant ($p<0.05$) correlation was observed between Pb and Zn in common carp while between Cu and Cd in silver carp. The concentration of heavy metals was in the suggested permissible limits and poses no threat if consumed. In order to maintain the heavy metals level within permissible limits, proper care should be taken along with regular assessment.

Key words: Fish consumption, heavy metals, health risk, WHO permissible limits

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Pakistan is witnessing a swift industrial and agricultural growth as well as a rapid increase in population. Effluents from industries, fertilizers from agriculture sector and different other anthropogenic activities lead to pollution (Ullah *et al*., 2016). Pollution due to use of different pesticides such as cypermethrin, deltamethrin, malathion, endosulfan and karate etc. and different heavy metals enormously deteriorate the environment. Heavy metals of these pollutants are dispersed during its production, recycling, its by-products manufacturing and recycling and ultimate disposal to the environment (Ullah, 2015). These render different biospheres such as aquatic bodies unfit for aquatic organisms, fish being the most eminent. In severe cases it leads to fish mortalities but in permissible range it does lead to bioaccumulation. Moreover, it also leads to oxidative stress, teratogenic or carcinogenic effects and damage DNA as well (Ullah and Zorriehzahra, 2015). When consumed these accumulated and biomagnified heavy metals result in serious implication in human beings.

It is a well-established fact that heavy metals' pollution is the key factor for contaminating our environment and foodstuffs (Gholizadeh *et al*., 2009; Khairy, 2009). This problem has been more severe in developing countries such as, Pakistan. Heavy metals that are more important on account of posing a serious threat to life or having the potential of increasing health risk for human are arsenic, aluminium, lead, cadmium, copper, mercury, chromium, manganese and antimony etc. (Mahmoud and Abdel-Mohsein, 2015). Cadmium and lead easily accumulate in food chains (Demirezen and Uruc, 2006) and get absorbed easily from the digestive tract and the atmosphere (Krejpcio and Trojanowska, 2000).

Absorption of these heavy metals in higher concentration lead to serious complications in humans such as Cd exposure results in endocrine disruption and can possibly lead to prostate cancer, breast cancer, kidney impairment and skeletal damage (Nordberg *et al*., 2002; Saha and Zaman, 2013) and Pb exposure leads to problems associated with the kidneys and nervous system and inhibits heme synthesis etc. (Berny *et al*., 1994). Owing to the importance of heavy metals in edibles, the present study was aimed to measure heavy metals' concentration in the muscle tissues of two widely consumed fish species, silver carp (*Hypophthalmichthys molitrix*) and common carp (*Cyprinus carpio*) collected from the market and hotels of district Lower Dir, Khyber Pakhtunkhwa, Pakistan. Concentration of heavy metals were investigated in the muscle tissues of the fish on account of being edible part. Moreover, it is considered as the main important tissue for the assessment of concentration of heavy metals in fish (Yousafzai and Shakoori, 2006). Therefore, it was felt necessary to assess the concentration of heavy metals in these fish species as the local masses consume a massive quantity of these edible fish species.

MATERIALS AND METHODS

Study area: Lower Dir district (34°, 37' to 35°, 07' North longitude and 71°, 31' to 72°, 14' East latitude) is one of the seven districts in Malakand division situated in hindukush range, shown in Fig. 1 (Ullah and Ahmad, 2015). Having an altitude of 2700 ft from sea level, bounded by Bajaur Agency (FATA) and Afghanistan (West), Swat district (East), Malakand (South) and Chitral district (North) (Ullah *et al*., 2014). Lower Dir is politically divided into seven tehsils namely; Balamabat, Timergara, Adenzai, Khall, Munda, Lal Qila and Samarbagh (Ullah and Nabi, 2015). Despite having different occupational castes, Pashto/Pushto is the sole language.

Sampling and heavy metals estimation: Nine specimens of *Cyprinus carpio* and *Hypopthalmichthys molitrix* were collected from three major markets of the district, namely Chakdara (tehsil Adenzai), Timergara (tehsil Timergara) and Khall (tehsil Khall). These places were selected on the basis of their large inhabitants. The collected specimens, transported to lab in ice box were washed and samples from muscles tissues were extracted out. The required portion of the tissues were weighed and were stored (20°C) for metals analysis.

The frozen thawed, rinsed, blotted and washed muscles tissues were transferred to volumetric flask for heavy metals analysis. Heavy metals including manganese (Mn), lead (Pb), iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd) and cobalt (Co) in the muscles of the fish species were measured using atomic absorption spectrophotometer (Z-2000 Hitachi) following standard protocol and procedure as followed by Ahmad *et al*. (2015).

Statistical analysis: Data obtained from experiment were expressed as Mean±SE. The result was analyzed by using one way analysis of variances (ANOVA) followed by Tukey-HSD test using statistix version 8.1. Values of $p<0.05$ were considered statistically significant. Map of the study area was prepared using ArcGIS 9.3 platform.

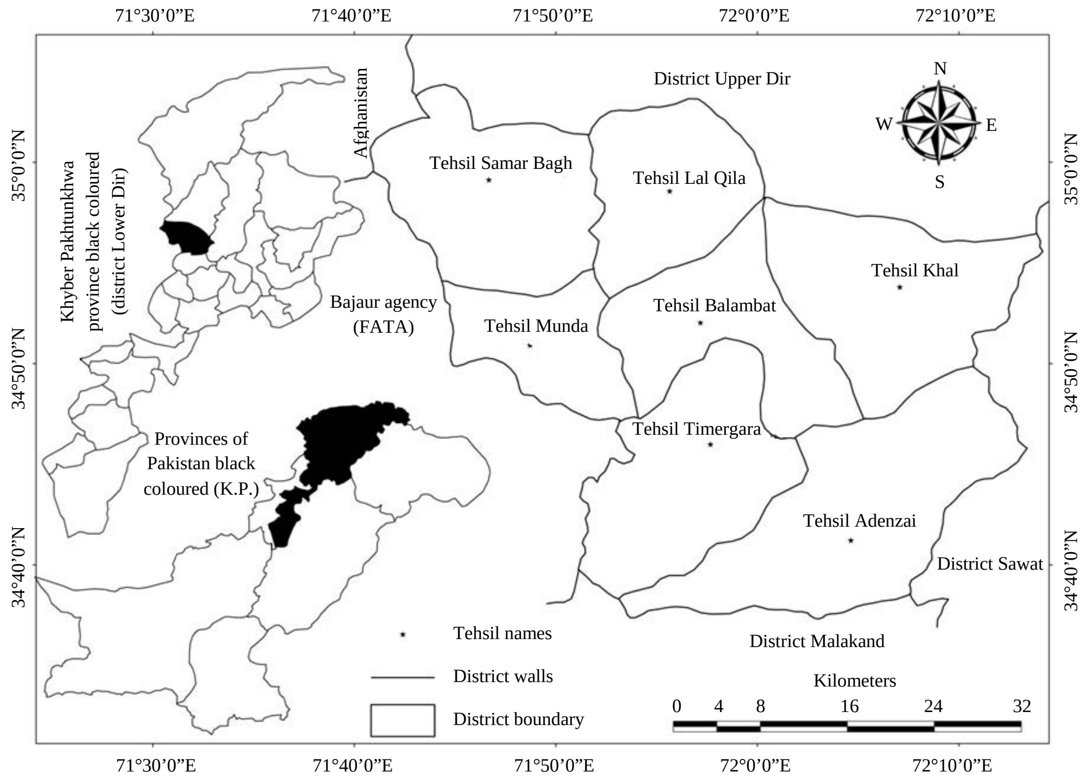

Fig. 1: Map showing political division of the study area, Lower Dir district

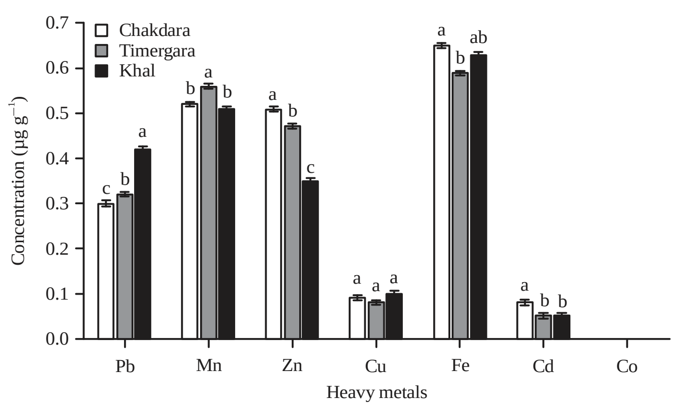

Fig. 2: Concentration (µg g^{-1}) of heavy metals in muscle tissue of common carp. Data are presented as Mean±SE (n = 9). Means with different letters are significantly different ($p<0.05$)

RESULTS

Heavy metals in *Cyprinus carpio*: In common carp the order of accumulation of heavy metals was varying for the study sites. The Pb was in order of Khal>Timergara>Chakdara, Mn was Timergara>Chakdara<Khal, Zn was Chakdara> Timergara>Khal, Cu was Khal>Chakdara>Timergara, Fe was Chakdara>Khal>Timergara and Cd was Chakdara>Timergara >Khal. Mean accumulation of heavy metals across study area was Fe>Mn>Zn>Pb>Cu>Cd. Figure 2 shows heavy metal concentration in common carp. Table 1 shows correlation coefficient matrix of the studied metals in common carp.

Heavy metals in *Hypophthalmichthys molitrix*: In silver carp the order of accumulation of Pb was Khal>Timergara = Chakdara, Mn Chakdara>Timergara>Khal, Zn Chakdara> Khal>Timergara, Cu Timergara>Chakdara>Khal, Fe was Timergara>Chakdara>Khal and Cadmium was in order of

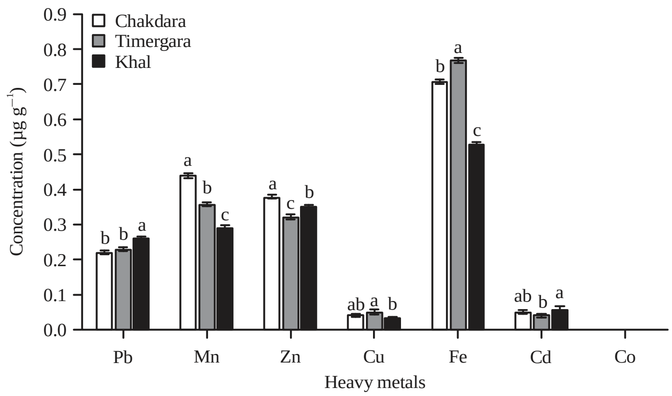

Fig. 3: Concentration (μg g^{-1}) of heavy metals in muscle tissue of silver carp. Data are presented as Mean±SE (n = 9). Means with different letters are significantly different (p<0.05)

Table 1: Correlation coefficient matrix of the studied heavy metals in common carp

Heavy metals	Pb	Mn	Zn	Cu	Fe	Cd
Pb	1					
Mn	-0.5291					
p-value	0.6451	1				
Zn	-0.9963	0.4539				
p-value	0.0510	0.7000	1			
Cu	0.7777	-0.9449	-0.7206			
p-value	0.4328	0.2123	0.4878	1		
Fe	0.0339	-0.8660	0.0524	0.6547		
p-value	0.9784	0.3333	0.9666	0.5456	1	
Cd	-0.6286	-0.3273	0.6934	0.0000	0.7559	
p-value	0.5672	0.7877	0.5122	1.0000	0.4544	1

r-values>0.5 are significant at p<0.05

Table 2: Correlation coefficient matrix of the studied heavy metals in silver carp

Heavy metals	Pb	Mn	Zn	Cu	Fe	Cd
Pb	1					
Mn	-0.9494					
p-value	0.2034	1				
Zn	-0.2402	0.8456				
p-value	0.5329	0.6422	1			
Cu	-0.7206	0.4663	-0.5000			
p-value	0.4878	0.6912	0.6667	1		
Fe	0.8846	0.6934	-0.2402	0.9608		
p-value	0.3088	0.5122	0.8456	0.1789	1	
Cd	0.7206	-0.4663	0.5000	-1.0000	-0.9608	
p-value	0.4878	0.6912	0.6667	0.0000	0.1789	1

r-values>0.5 are significant at p<0.05

Khal>Chakdara>Timergara. The mean concentration of heavy metals across the sampling markets was Fe>Mn>Zn>Pb>Cd>Cu. Figure 3 shows the concentration of the heavy metals in silver carp. Table 2 shows correlation coefficient matrix of the studied metals in silver carp.

DISCUSSION

Fish is capable of accumulating high amount of metals in its tissues. These metals might be cationic complexes in nature and concentrated in fish tissues. It is well established from previous research that fish can biomagnify toxic metals, specifically least water soluble ones. This might be due to the close contact of the sources bringing these toxic metals in solution or suspension form. Moreover, fish uses water soluble oxygen through filtering massive water quantity through the gills (Yousafzai *et al*., 2014). Metals are biomagnified in various tissues of the fish in different concentrations, which depend on different factors, including physico-chemical characterization of the water, season and level of availability of heavy metals in ambient water (Kargin and Erdem, 1991).

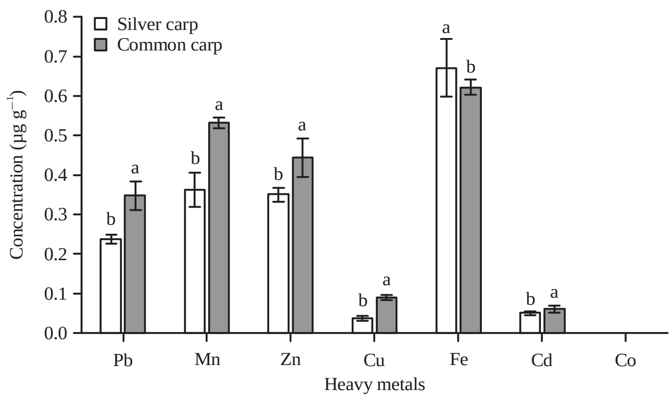

Fig. 4: Comparative heavy metals' concentration (µg g^{-1}) in common carp and silver carp. Data are presented as Mean±SE (n = 9). Means with different letters are significantly different ($p<0.05$)

The results of the current study revealed different degrees of accumulation of heavy metals studied in both the species even the specimens of the same fish sampled from three different markets. There was a statistical significant ($p<0.5$) difference between both the species and same heavy metals for the study sites. Cobalt was not detected throughout the study area in any of the specimens while the rest of the metals were detected in varying amounts in different specimens and sampling markets.

Concentration of the heavy metals was varied across sampling sites and were statistically significant ($p>0.5$). Both the species showed various levels of accumulation of different heavy metals such as, Pb, Mn, Zn, Cu and Cd were higher in common carp whereas Fe was higher in silver carp. This may be attributed to the large size of the specimens of common carp collected which might have accumulated higher concentration of the studied metals. This might also be due to the fact that common carp would have been purchased from an area or fish farm having a higher amount of heavy metals in its sediments and water. The level of heavy metals in ambient water and sediments is directly associated with higher absorption of heavy metals by the fish. Figure 4 shows the comparative concentration of heavy metals in both the species.

The mean concentration of Pb in common carp was 0.347±0.037, Mn 0.530±0.015, Zn 0.443±0.048, Cu 0.090±0.006, Fe 0.623±0.018 and Cd was 0.060±0.011 µg g^{-1} (Fig. 2). Statistically significant correlation was observed between Pb and Zn. Table 1 shows a correlation coefficient matrix for the studied heavy metals in common carp. The mean concentration of Pb, Mn, Zn, Cu, Fe and Cd in silver carp was 0.237±0.012, 0.363±0.043, 0.350±0.017, 0.040±0.006, 0.670±0.072 and 0.050±0.006 µg g^{-1}, respectively (Fig. 3). The correlation between Cu and Cd was statistically significant in silver carp. Table 2 shows a correlation coefficient matrix for the studied heavy metals in silver carp.

All the studied heavy metals were in the permissible limits suggested by FAO (1983), FAO/WHO (1989) and ITS (2000) for food/fish consumption and were less than earlier reported in different tissues of wild fish from different parts of the province (Yousafzai and Shakoori, 2006; Yousafzai *et al*., 2009; Yousafzai *et al*., 2010; Yousafzai *et al*., 2012; Iqbal and Shah, 2014; Yousafzai *et al*., 2014; Ahmad *et al*., 2015), other provinces of the country (Korai *et al*., 2008; Jabeen *et al*., 2012; Tabinda *et al*., 2013; Malik *et al*., 2014) and different countries around the globe (Amundsen *et al*., 1997; Carvalho *et al*., 2002; Canli and Atli, 2003; Yilmaz, 2003; Carvalho *et al*., 2005; Demirak *et al*., 2006; Dural *et al*., 2007; Al-Ghanim *et al*., 2015). This might be due to the fact that these fish species would have been transported from an area or fish farm having lower concentration of heavy metals in its water and sediments.

The concentration of heavy metals in the muscle tissues of *Cyprinus carpio* and *Hypophthalmichthys molitrix* were also within the suggested permissible limits suggested by different agencies including canadian standards (Zn and Cu; 100 µg g^{-1}), UK (Pb; 1 µg g^{-1}), USEPA (Ni; 1 µg g^{-1}), ITS (Cd; 0.1 µg g^{-1}, Zn; 50 µg g^{-1}) (Korai *et al*., 2008; Aktan and Tekin-Ozan, 2012; Tabinda *et al*., 2013). The accumulation of heavy metals in both the fish species was much lesser that the previous studies conducted on various species including *Tor putitora* (Yousafzai *et al*., 2009), *Wallago attu* and *Labeo dyocheilus* (Yousafzai *et al*., 2010), *Cyprinus carpio* (Yousafzai *et al*., 2012; Iqbal and Shah, 2014), *Cirrhinus mrigala* (Tabinda *et al*., 2013), *Tor putitora, Cirrhinus mrigala, Labeo calbasu* and *Channa punctatus* (Malik *et al*., 2014) and *Wallago attu, Aorichthys seenghala, Cyprinus carpio, Labeo dyocheilus* and *Ompok bimaculatus* (Ahmad *et al*., 2015).

Higher concentration of heavy metals reported in previous studies might be attributed to the higher amount of contamination in their collections sites and reservoirs due to indiscriminate dumping of industrial and municipal effluents, as surface water pollution is regarded as the major source of accumulation of heavy metals in fish (Singh *et al*., 2014).

CONCLUSION

From the results of the current study, it was concluded that there is no threat using both the fish species available in markets in the study area. Although, fish is highly used in the study area yet no health risk has been reported or identified in the study area which is in confirmation with the results of the current study. To maintain good quality of fish meat, free of all heavy metals, environmental protection agencies should play their role and should implement strict role regarding dumping and disposition of heavy metals or its product as well as any material having heavy metals in higher amount. Mass awareness at the local level should be prioritized as alteration of fish meat quality might be observed in future on account of heavy metals use in enormous amount in industries, domiciliary chores and agricultural practices in the province.

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